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The Panamanian Aotus - Plasmodium falciparum model was used to evaluate antimalarial drugs, singly and in combination. Azithromycin cured Smith/RE strain infections. Tetrandrine (WR 149544) plus chloroquine reversed chloroquine resistance in vivo as indicated only by parasite clearance, not infection cure. WR 99210 retained its antimalarial activity when coadministered with folinic acid, a combination that may ameliorate toxicity of WR 99210. Results of trials (still current) to induce resistance to WR 250417 and WR 169626 show that such resistance may have occurred by treatment with subcurative doses and parasite subinoculations. A chloroquine-resistant P. vivax strain was adpated to Panamanian Aotus; drug resistance was confirmed. A chloroquine-sensitive P. vivax strain was not infective for unaltered Panamanian Aotus.

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Richard V. Bozzan 15 March 1994

PI Signature Date

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INTRODUCTION

The essence of the problem addressed in this report is to evaluate the potential antimalarial activity of drugs in the pre-clinical model of Aotus lemurinus lemurinus (Panamanian night monkey) experimentally infected with Plasmodium falciparum. Such studies with this model were initiated in 1976 at Gorgas Memorial Laboratory, Panama and supported, in part, by the U.S. Army Medical Research and Development Command. Due to the drug resistance exhibited by the highly pathogenic P. falciparum parasites in Asia, Africa; and Latin America, it is essential that new drugs be evaluated in the preclinical Aotus model for their potential usefulness against human infections.

Initially, antimalarial drug studies used the Colombian Aotus as the experimental host (1,2). In the mid 1970's embargoes imposed by South American countries on the exportation of monkeys seriously restricted the use of Aotus for biomedical research in the United States. Panamanian Aotus were available at Gorgas Memorial Laboratory, Panama, and the project transferred here in 1976. Diverse avenues of research have been pursued in attempts to identify effective new antimalarial drugs. Three strains of P. falciparum, Vietnam Smith, Uganda Palo Alto, and Vietnam Oak Knoll, had been adapted to Panamanian Aotus. These strains exhibit diverse susceptibility and/or resistance to standard antimalarial agents. The course of untreated infections in Panamanian Aotus has been characterized and compared with that in Aotus of Colombia (3). Overall, the virulence of these strains was less in Panamanian than in Colombian owl monkeys, as indicated by lower mortality rates of Panamanian monkeys during the first 30 days of patency. Maximum parasitemias of the Vietnam Smith and Uganda Palo Alto strains were, however, significantly higher during the first 15 days of patency in Panamanian than in Colombia owl monkeys. These quantitative differences in infection parameters between Panamanian and Colombian owl monkeys have not invalidated the use of the former for the evaluation of new antimalarial drugs.

Numerous candidate antimalarial drugs of diverse chemical classes have been evaluated against trophozoite-induced infections of one or more P. falciparum strains during the course of these contracts. In seeking alternatives to primaquine, two 8-aminoquinolines proved to be active aganist the blood stages of P. falciparum (4,5). Desferrioxamine, an iron-specific chelating agent, was shown to suppress parasitemias of the virulent Uganda Palo Alto strain of P. falciparum (6). The in vitro activity of two halogenated histidine analogs was not confirmed by evaluation against P. falciparum infections in owl monkeys (7).

Chloroquine-resistance of P. falciparum represents the

the greatest challenge in developing effective antimalarial drugs. Reversal of chloroquine-resistance in P. falciparum, in vitro, was achieved by the co-administration of verapamil (a calcium channel blocker) plus chloroquine (8). Other in vitro studies have shown that there is a significantly greater efflux of chloroquine from erythrocytes containing falciparum parasites resistant to chloroquine than from red cells parasitized by chloroquine-sensitive falciparum malaria (9). Calcium channel blockers appear to prevent this active efflux of chloroquine, thus allowing the drug to accumulate to parasiticidal levels.

Based upon the success of in vitro reversal of chloroquine-resistance, trials were initiated to determine if resistance could be reversed in Aotus infected with the chloroquine-resistant Vietnam Smith strain of P. falciparum. Six calcium channel blockers, or similarly acting drugs, were co-administered with chloroquine in diverse regimens. The desideratum of chloroquine-resistance reversal was administration of a single course of treatment, with parasite clearance and infection cure. Suppression of parasitemia was obtained during an initial course of treatment, but parasite clearance and cure occurred in some instances only after re-treatment. Such infection parameters were similarly to those in monkeys with self-limited infections and cure could be attributed to acquired immunity.

Limited trials with desipramine, Norpramin, a tricyclic psychotropic drug, demonstrated the feasibility of reversing chloroquine-resistance in vivo (10). Parasite clearance was obtained, but the infection was not cured.

Subsequently, in vivo reversal of chloroquine resistance was obtained with combinations of chloroquine plus chlorpromazine or prochlorperazine. Such reversal was exhibited by rapid suppression and clearance of parasitemia, resulting in infection cure without retreatment (11).

Evaluation of two oil-soluble derivatives of artemisinin, artemether and arteether, demonstrates that both possess similar activity to cure infections of a multi-drug resistant P. falciparum strain in Aotus.

Both the purpose and methods of approach of the present work remains essentially unchanged since 1976, viz to ascertain the antimalarial activity of drugs against P. falciparum infections in Aotus. The method of approach may vary on an ad hoc basis, such as administering a combination of drugs.

BODY

I. Experimental Methods

The general intent of this project is to evaluate the potential antimalarial activity of drugs, or combination thereof, in the preclinical model of Aotus experimentally infected with P. falciparum (or P. vivax). Specifically, the vertebrate host is Aotus lemurinus lemurinus, the Panamanian night monkey. These animals are either feral, laboratory adapted or laboratory born. No naturally acquired, human plasmodium infection has been reported in Aotus. The Vietnam Smith/RE strain of P. falciparum was adapted to Aotus of Colombian origin in 1971 (1) and in Panamanian Aotus in 1976. (3) The course of untreated infections, essential for comparison with treated infections, has been documented in Panamanian Aotus (3). This plasmodium strain is resistant to maximally tolerated doses of chloroquine, pyrimethamine, and quinine (2).

Cryopreserved samples of two P. vivax strain were received from LTC G. Dennis Shanks, Army Malaria Research Unit, Ingleburn, Australia. The New Guinea AMRU-1 strain is resistant to chloroquine, while the AMRU-2 strain is chloroquine sensitive. These strains were to be adapted to Panamanian Aotus, their response to chloroquine evaluated, and then tested against WR 238605. Details of the adaptation procedures and drug response will be presented in the appropriate section of this report.

To initiate an experiment, infected blood (with 2.5% sodium citrate as the anticoagulant) from an untreated Aotus was diluted appropriately in chilled saline (0.85%), such that each milliliter contained 5,000,000 parasites. This amount was inoculated into the saphenous vein of experimental and control monkeys.

Blood films, prepared and examined daily beginning on the first post-inoculation day, were stained with Giemsa. Parasitemias were evaluated as follows: negative, if no parasites were detected on a thick blood film after examination for at least 5 minutes; <10 parasites per cmm, if positive only on the thick blood film; parasite enumeration was by the Earle-Perez method and reported as the number of parasites per cmm. (12)

Blood films from untreated Aotus, serving as passage and/or control subjects, were prepared and examined daily during the primary patent period, and daily thereafter for at least three consecutive days after parasites could last be detected on thick blood films. When parasitemia had cleared, films were made and examined twice weekly until a total of 100 negative days had been recorded. If a recrudescence occurred, blood films were obtained again on a

daily basis.

Parasitemias were evaluated daily during the treatment period and until blood films were negative for at least seven consecutive days. The frequency of smearing was then reduced to two times per week (Monday and Thursdays or Tuesdays and Fridays). If no recrudescences occurred during a 100 day examination period, the infection was considered to have been cured.

Drug doses were calculated as mg base per kg of body weight. Stock solutions of water soluble compounds, at appropriate concentrations, were prepared with distilled water and stored at 8 C for the treatment period. If a compound was water insoluble, a suspension of the requisite amount of drug was prepared daily with 0.3% methylcellulose (in distilled water).

Oral administration of drugs was by gastric intubation with a 14 French catheter. The total volume of fluid administered, drug solution or suspension, and rinse was 14 ml.

As will be indicated, some drugs were administered intramuscularly.

II. Results

A. WR 279377AC (BM 16640), azithromycin

WR 100553AA (ZM 33452), doxycycline

Among the antibacterial antibiotics, both tetracycline and doxycycline are effective against drug resistant P. falciparum infections. Althogh erythromycin is inactive against chloroquine-resistant falciparum infections, an analogue, azithromycin, is effective in vitro against P. falciparumand against P. berghei in the mouse model. The study reported here compares the activities of WR 279377, azithromycin and WR 100553, doxycycline against infections of the multi drug resistant Vietnam Smith/RE strain of P. falciparum.

Detailed antimalarial activity is presented in Table 1 and summarized in Tables 2 and 3. A 30.0 mg/kg dose of azithromycin administered for 7 days cleared parasitemia (with recrudescence) in one Aotus while parasitemia was only suppressed in another animal. The same primary regimen of doxycycline cleared parasitemia in each of two monkeys, but did not cure infection, although this regimen against azithromycin treatment failures did cure infections. Primary treatment with azithromycin at a 100.0 mg/kg dose for 7 days cured infections in 2 of 2 Aotus.

B. WR 149544AC (BM 17594), tetrandrine

WR 1544BM (AR 20613), chloroquine

In a previous study to reverse chloroquine resistance in vivo, co-administration of tetrandrine (15.0 mg/kg/7 days) and chloroquine (20.0 mg/kg/7 days) cleared Vietnam Smith/RE parasitemias in 3 of 3 Aotus, but did not cure infections. Retreatment with tetrandrine (30.0 mg/kg/7 days) and chloroquine (20.0 mg/kg/7 days) cleared parasitemia in each of two monkeys; the infection in one monkey recrudesced, and the infection in the other animal was cured. The desideratum for in vivo reversal of chloroquine resistance is infection cure following primary treatment since cure after retreatment is a combination of drug activity plus acquired immunity.

Results of additional trials to reverse chloroquine resistance in vivo are detailed in Table 4, and summarized in Tables 5 and 6. Primary treatment with tetrandrine (30.0 and 60.0 mg/kg/7 days) plus chloroquine (20.0 mg/kg x 7 days) cleared parasitemia with recrudescence in two Aotus. Retreatment with twice respective dose of tetrandrine administered during the primary treatment plus the daily maximum tolerated dose of chloroquine (20.0 mg/kg) cleared parasitemia, but without cure.

Considering the possibility that a 7 day course of treatment was not sufficient to cure infections, the two monkeys were administered tetrandrine (25.0 mg/kg) plus chloroquine (20.0 mg/kg) for 14 days. This was the third drug regimen each monkey had received. Blood films in one Aotus (12705rr) were parasite negative at the time treatment was initiated. No recrudescence was observed during the post treatment observation period.

C. WR 99210 AD (AW 23628)

WR 139004AC (BK 64208), folinic acid

The objective of this experiment was to determine if the toxicity of WR 99210 could be obviated by the co-administration of folinic acid and still retain its antimalarial activity against infections of the multi-drug resistant Smith/RE strain of P. falciparum. Parasite response is detailed in Table 7 and summarized in Tables 8 and 9.

WR 99210 administered alone, cleared parasitemias (without cure), at doses of 10.0 and 20.0 mg/kg x 7 days. Primary treatment with WR 99210 (10.0 mg/kg/7 days) plus folinic acid (0.33 mg/kg/7 days) cleared parasitemia in 2 of 2 Aotus but with recrudecence. The same regimen of WR 99210 but with 1.0 mg/kg/7 days of folinic acid cleared

parasitemia in each of two monkeys, curing the infection in one subject. As shown in the tables, re-treatments cured infections in 2 of 3 Aotus.

D. WR 250417AG (BN 34278)

WR 169626AC (BK 09350)

While these drugs, both pyrimethamine analogues have shown some activity against a pyrimethamine-resistant strain (Smith/RE) of P. falciparum in Aotus, there was some indication that resistance was induced by repeated re-treatments. This present experiment was designed to determine specifically if resistance could be generated rapidly. For each drug, two infected animals each were administered a subcurative (or suppressive) dose and two infected monkeys administered a putative curative dose. Following administration of the lowest dose, if parasite suppression, or clearance with recrudescence occurred, parasites were subinoculated into a malaria naive Aotus and both donor and recipient treated with the next highest dose. If treatment failure occurred, then a second subinoculation was done, with donor and recipient being administered the putative curative dose. Drugs were given intramuscularly to diminish drug utilization and obviate any absorption problems.

Detailed parasite responses to WR 250417 are shown in Table 10, and summarized in Table 11. Subinoculation lines and treatments with increased doses are depicted in Figure 1. A 0.25 mg/kg(x3) dose suppressed parasitemia in Aotus 12719, parasites were subinoculated to 12718, and both donor and recipient monkeys treated with a 0.75 mg/kg(x3) dose. Parasites were cleared in 12719r, and blood films have remained negative for 57 days. The parasitemia in 12718 was suppressed by a dose of 0.75 mg/kg(x3), but cleared by retreatment with a 2.5 mg/kg(x3) dose, with blood films negative for 34 days, to date.

A second parasite line, possibly resistant to WR 250417, was initiated following a 0.25 mg/kg(x3) dose administered to Aotus 12720. Parasites, subinoculated to 12717, were cleared (with recrudescence) by a dose of 0.75 mg/kg(x3), as were those in the donor 12717. Retreatment of recrudescent parasites in 12717r with a 2.5 mg/kg(x3) dose cleared parasites, and blood films have been negative for 29 days. Recrudescent parasites, subsequent to treatment with a dose of 0.75 mg/kg(x3) in 12720r, were subinoculated to 12701; both donor and recipient administered a dose of 2.5 mg/kg(x3). Parasitemia was suppressed in the recipient (12701), and retreated with 10.0 mg/kg(x3) dose, resulting in clearance with recrudescence. Following administration of a 2.5 mg/kg(x3) dose to the donor (12720rr), parasites were cleared (with

recrudescence), and retreated with a 10.0 mg/kg/(x3) dose, resulting in parasite clearance, blood films negative for 26 days.

As shown in Figure 2, WR 169626 administered at a dose of 0.25 mg/kg(x3) to Aotus 12684, cleared parasitemia, and recrudescent parasites subinoculated to 12681. Parasites in both donor and recipient were then treated with 0.75 mg/kg(x3), clearing parasites in the donor (12684) for 45 days, to date. In contrast, a recrudescence occurred in the subinoculee (12681), following administration of WR 169626 at a dose of 0.75 mg/kg(x3). Parasites were then subinoculated to 12674, and both donor and recipient administered a dose of 5.0 mg/kg(x3). This dose cleared parasites in the recipient (12674), blood films remaining negative for 33 days, to date. Parasites were cleared (with recrudescence) in the donor (1281r), and retreated with 10.0 mg/kg(x3).

A dose of 0.25 mg/kg(x3) cleared parasitemia in 12686, recrudescent parasites subinoculated to 12725, and both donor and recipient monkeys treated with a dose of 0.75 mg/kg(x3). This dose cleared parasites (for at least 45 days) in the donor (12686r), while the recrudescence in 12725 was treated with 5.0 mg/kg(x3), blood films remaining negative for at least 22 days.

E. Plasmodium vivax

1. Adaptation to Panamanian Aotus.

A cryopreserved sample of two strains of P. vivax were received from LTC G. Dennis Shanks, Army Malaria Research Unit, Ingleburn, Australia: New Guinea AMRU-1 (chloroquine resistant, from the 10th Aotus passage) and New Guinea AMRU-2 (chloroquine sensitive, 1st Aotus passage). These parasite strains were to be adapted to Panamanian Aotus, infection parameters characterized, confirm their response to chloroquine, and then expand the evaluation of WR 238605, a primaquine analogue against infections.

Each cyopreserved sample was thawed rapidly under cold, running tap water and inoculated intraperitoneally into a splenectomized monkey. All monkeys used for P. vivax studies are cured of P. falciparum infection; a patent infection of the AMRU-1 (CQR) strain began on day 15 post inoculation, while parasites of the AMRU-2 (CQS) strain were first detected on day 13 post inoculation. Parasites of each strain were then subinocualted into a second splenectomized Aotus.

Infection parameters during adaptation of the AMRU-2 (CQS) strain are summarized in Table 14. Although these parasites developed in splenectomized monkeys, it was not

possible to adapt them to unaltered hosts. An inoculum of 65 x 10^6 parasites was completely ineffective in producing a patent infection.

In contrast, the AMRU-1 (CQR) strain adapted readily to normal monkeys subsequent to the second passage in a splenectomized animal (Table 15). A standard inoculum of 5x10^6 parasites produced reproducible infections. At the 13th serial passage, and experiment was initiated to confirm chloroquine resistance of the AMRU-1 strain of P. vivax.

2. WR 1544BM (AR 20613), chloroquine.

Detailed response of the AMRU-1 strain to chloroquine are given in Tables 16 and 17, while summarized in Tables 18, 19 and 20. Total chloroquine doses of 17.5, 35.0, and 30.0 mg/kg, whether administered over 7 days or 3 days, produced either no response or suppressed parasitemia. These results conform to chloroquine resistance.

III. Discussion

Azithromycin (30.0 mg/kg x 7 days) was less effective than doxycycline at the same regimen against the multidrug Vietnam Smith/RE strain of P. falciparum. Azithromycin (100.0 mg/kg x 7 days), however, did cure infections in 2 of 2 Aotus. Despite the fact that parasite clearance was relatively slow (14 and 10 days, respectively), these data suggest that azithromycin may replace drugs of the tetracycline class as effective blood schizonticides of drug resistant falciparum infections.

As in the initial trial with tetrandrine (WR 149557) to reverse chloroquine resistance in vivo when co-administered with chloroquine, the experiment herein reported yielded similar results, in that parasites were cleared only. Said clearance occurred subsequent to primary and one retreatment for seven days. Although following a 14 day treatment no recrudescence was observed, it must be recognized that this regimen was initiated on the 109th day after inoculation, and that blood films were negative in one monkey at that time. Therefore, it cannot be stated with any assurance that a 14 day regimen of tetrandrine plus chloroquine, if administrated as a primary treatment, would cure infections of the chloroquine-resistant Vietnam Smith/RE strain.

Studies some 20 years ago showed that WR 99210, a dihydrofolate reductase inhibitor, was active against both pyrimethamine-sensitive and pyrimethamine-resistant strain of P. falciparum. Toxicity trials in humans produced significant gastro-intestinal symptoms such that phase I evaluation was terminated. One approach to decrease these drug-induced side effects was the co-administration of folinic acid without compromising antimalarial activity.

There were no animal data for this drug combination. Results of the single experiment contained in this report show that folinic acid plus WR 99210 does not decrease efficacy against infections of the pyrimethamine-resistant Smith/RE strain. Based upon these data, further human toxicity trials with the drug combination may be warranted.

Although the experiment to determine if resistance to WR 250417 and WR 169626 can be generated in Vietnam Smith/RE infection in Aotus is still in progress, results to date would indicate that such resistance has been induced to WR 169626. This conclusion is based on the fact that a 5.0 mg/kg (x 3 day) dose appears to be curative. The infection in one monkey, in a subinoculation line, recrudesced subsequent to be treated with the putative curative dose. For WR 250417, the primary treatment dose of 2.5 mg/kg (x3 days) has proven not to be curative. Yet the parasitemia in Aotus 12701 (a subinoculee) was suppressed only by those drug dose, and cleared, with recrudescence, by 10.0 mg/kg (x3 days). Moreover, repetitive treatment plus acquired should have produced more potential infection cures than are indicated in the data.

The chloroquine-resistant New Guinea AMRU-1 strain of P. vivax was adapted to normal (spleen intact) Aotus after the second passage in splenectomized monkeys. Consecutive blood passage in 13 Aotus yielded reproducible infection parameters, such that it was feasible to confirm the strains' resistance to chloroquine. The drug regimens used have cured chloroquine-sensitive P. vivax infections.

Despite 9 serial passages of the chloroquine-sensitive AMRU-2 strain of P. vivax in splenectomized Aotus, the parasites remained non-infective for normal monkeys. No explanation for this lack of infectivity is forthcoming, as P. vivax has proven to be readily adaptable to Panamanian Aotus. Parasites were cryopreserved for future adaptation trials.

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TABLE 1

DETAILED ACTIVITY OF WR 279377AC(BM 16640), AZITHROMYCIN AND WR 100553AA (ZM 33452), DOXYCYCLINE, AGAINST INFECTIONS OF THE VIETNAM SMITH/RE STRAIN OF PLASMODIUM FALCIPARUM

Aotus No.	Daily Dose Mg/Kg	Parasitemia per cmm x 10^3									
		Day Pre-Rx	Day of Treatment							Day Post Treatment		
			1	2	3	4	5	6	7	1	2	3
12689	30.0a	10	26	122	419	401	69	6	3	0.1	<0.01	0
12690	30.0a	6	17	99	394	145	40	2	0.3	0.1	<0.01	<0.01
12695	100.0a	3	1	62	112	121	117	66	11	5	1	0.3
12691	100.0a	2	2	48	124	31	13	0.7	0.2	0.03	<0.01	0
12699	30.0b	5	1	140	299	148	41	3	0.2	0.04	<0.01	0
12700	30.0b	4	2	93	202	112	39	55	32	4	0.7	<0.01
12689r	30.0b	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0	0	0	0	0
12690r	30.0b	7	22	88	21	2	0.2	<0.01	0	0	0	0
12699r	85.0b	<0.01	<0.01	<0.01	1	0.5	<0.01	0	0	0	0	0
12700r	85.0b	<0.01	<0.01	0	0	0	0	0	0	0	0	0

a. Azithromycin
b. Doxycline

TABLE 2

SUMMARY OF THE ACTIVITY OF WR 279377AC (BM 16640), AZITHROMYCIN, AND WR 100553AA (ZM 33452), DOXYCLINE, AGAINST INFECTIONS OF THE VIETNAM SMITH/RE STRAIN OF PLASMODIUM FALCIPARUM

Monkey No.	Dose x 7 Mg/Kg	Response of Parasitemia to Rx			Days from Initial Rx to Parasite Clearance	Days from Final Rx To Recru-descence	Notes
		None	Suppressed	Cleared			
12689	30.0a			+	10	8	Re-Rx, WR 100553
12690	30.0a		+		n.a.	n.a.	Re-Rx, WR 100553
12695	100.0a			+	14	n.a.	Cured
12691	100.0a			+	10	n.a.	Cured
12699	30.0b			+	10	14	Re-Rx, higher dose
12700	30.0b			+	12	12	Re-Rx, higher dose
12689r	30.0b			+	6	n.a.	Cured
12690r	30.0b			+	7	n.a.	Cured
12700r	85.0b			+	2	n.a.	Cured
12699r	85.0b			+	6	n.a.	Cured

a. azithromycin
b. Doxycline

TABLE 3

SUMMARY OF THE ACTIVITY OF WR 279337AC (BM 16640), AZITHROMYCIN, AND WR 100553AA (ZM 33452), DOXYCYCLINE, AGAINST PLASMODIUM FALCIPARUM

MALARIA	DOSE mg/kg		PRIMARY TREATMENTS		REPEAT TREATMENTS		TOTAL TREATMENTS	
STRAIN	TOTAL	DAILY	CLEARED	CURED	CLEARED	CURED	CLEARED	CURED
Smith/RE	210.0a	30.0	1/2	0/2			1/2	0/2
	700.0a	100.0	2/2	2/2			2/2	2/2
	210.0b	30.0	2/2	0/2	2/2	2/2	4/4	2/4
	595.0b	85.0			2/2	2/2	2/2	2/2

a. Azythromycin

b. Doxycycline

TABLE 4

DETAILED ACTIVITY OF WR 149557AC (BM 17594), TETRANDRINE, PLUS WR 1544BM (AR 20613) CHLOROQUINE AGAINST INFECTIONS OF THE VIETNAM SMITH/RE STRAIN OF PLASMODIUM FALCIPARUM

Aotus No.	Daily Dose Mg/Kg	Parasitemia per cmm x 10^3									
		Day Pre-Rx	Day of Treatment							Day Post Treatment		
			1	2	3	4	5	6	7	1	2	3
12705	30.0a 20.0b	4	4	72	24	16	1	<0.01	0	0	0	0
12706	60.0a 20.0b	0.9	1	24	5	0.5	<0.01	<0.01	0	0	0	0
12705r	60.0a 20.0b	5	8	13	5	2	0.4	<0.01	0	0	0	0
12706r	120.0a 20.0b	<0.01	<0.01	<0.01	1	0.9	<0.01	<0.01	0	0	0	0
12705rr	25.0a* 20.0b*	0	0	0	0	0	0	0	0	0	0	0
12706rr	25.0a* 20.0b*	<0.01	<0.01	<0.01	<0.01	0	0	0	0	0	0	0

a WR 149557
b WR 1544
* Daily dose for 14 days

TABLE 5

SUMMARY OF THE ACTIVITY OF WR 149557AC (BM 17594), TETRANDRINE, PLUS WR 1544BM (AR 20613), CHLOROQUINE AGAINST INFECTIONS OF THE VIETNAM SMITH/RE STRAIN OF PLASMODIUM FALCIPARUM

Monkey No.	Dose x 7 Mg/Kg	Response of Parasitemia to Rx			Days from Initial Rx to Parasite Clearance	Days from Final Rx To Recru-descence	Notes
		None	Suppressed	Cleared			
12705	30.0a 20.0b			+	7	9	Re-Rx, higher dose
12706	60.0a 20.0b			+	7	14	Re-Rx, higher dose
12705r	60.0a 20.0b			+	7	44	Re-Rx
12706r	120.0a 20.0b			+	7	16	Re-Rx
12705rr	25.0a* 20.0b			c	c	n.a	Cured
12706rr	25.0a* 20.0b			+	4	n.a.	Cured

* Daily dose for 14 days

c Blood films negative when treatment initiated

a. WR 149557
b. WR 1544

TABLE 6

SUMMARY OF ACTIVITY OF WR 149557AC(BM 17594), TETRANDRINE, PLUS WR 1544BM(AR 20613), CHLOROQUINE, AGAINST PLASMODIUM FALCIPARUM

MALARIA STRAIN	DOSE mg/kg TOTAL	DOSE mg/kg DAILY	PRIMARY TREATMENTS CLEARED	PRIMARY TREATMENTS CURED	REPEAT TREATMENTS CLEARED	REPEAT TREATMENTS CURED	TOTAL TREATMENTS CLEARED	TOTAL TREATMENTS CURED
Smith/RE	210.0a 140.0b	30.0 20.0	1/1	0/1			1/1	0/1
	350.0a 280.0b	25.0 20.0			1c/2	2/2	1c/2	2/2
	420.0a 140.0b	60.0 20.0	1/1	0/1	1/1	0/1	2/2	0/2
	840.0a 140.0b	120.0 20.0			1/1	0/1	1/1	0/1

a. WR 149557, tetrandrine

b. WR 1544, chloroquine

c. Blood films parasite negative in one Aotus when treatment was initiated

TABLE 7

DETAILED ACTIVITIES OF WR 99210AD (AW 23628) AND WR 139004AC (BK 64208), FOLINIC ACID, AGAINST INFECTIONS OF THE VIETNAM SMITH/RE STRAIN OF PLASMODIUM FALCIPARUM

Aotus No.	Daily Dose Mg/Kg	Parasitemia per cmm x 10^3										
		Day Pre-Rx	Day of Treatment							Day Post Treatment		
			1	2	3	4	5	6	7	1	2	3
12692	10.0a	4	2	57	18	1	<0.01	0	0	0	0	0
12692r	20.0a	0.9	13	5	0.8	0.3	<0.01	<0.01	0	0	0	0
12693	10.0a 0.33b	2	2	54	14	0.5	<0.01	0	0	0	0	0
12694	10.0a 0.33b	3	3	42	3	0.5	<0.01	<0.01	0	0	0	0
12703	10.0a 1.0b	7	13	57	13	1	<0.01	<0.01	0	0	0	0
12704	10.0a 1.0b	4	16	26	2	0.6	<0.01	<0.01	0	0	0	0
12693r	10.0a 1.0b	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0	0	0	0	0
12694r	10.0a 1.0b	6	17	1	<0.01	<0.01	0	0	0	0	0	0
12703r	20.0a 1.0b	5	12	0.9	<0.01	<0.01	0	0	0	0	0	0

a. WR 99210

b. WR 139004

TABLE 8

SUMMARY OF THE ACTIVITIES OF WR 99210AD(AW 23628) AND WR 13900AC (BR 64208), FOLINIC ACID, AGAINST INFECTIONS OF THE VIETNAM SMITH/RE STRAIN OF PLASMODIUM FALCIPARUM

Monkey No.	Dose x 7 Mg/Kg	Response of Parasitemia to Rx			Days from Initial Rx to Parasite Clearance	Days from Final Rx To Recru-descence	Notes
		None	Suppressed	Cleared			
12692	I0.0a			+	6	6	Re-Rx, higher dose
12692r	20.0a			+	7	21	
12693	10.0a 0.33b			+	6	13	Re-Rx, higher dose
12694	10.0a 0.33b			+	7	18	Re-Rx, higher dose
12703	10.0a 1.0b			+	7	17	Re-Rx, higher dose
12704	10.0a 1.0b			+	7	n.a.	Cured
12693r	10.0a 1.0b			+	6	12	
12694r	10.0a 1.0b			+	5	n.a.	Cured
12703r	20.0a 1.0b			+	5	n.a.	Cured

a. WR 99210 b. WR 139004

TABLE 9

SUMMARY OF THE ACTIVITY OF WR 99210AD (AW 2368) AND WR 139004AC (BK 64208), FOLINIC ACID AGAINST PLASMODIUM FALCIPARUM

MALARIA STRAIN	DOSE mg/kg TOTAL	DOSE mg/kg DAILY	PRIMARY TREATMENTS CLEARED	PRIMARY TREATMENTS CURED	REPEAT TREATMENTS CLEARED	REPEAT TREATMENTS CURED	TOTAL TREATMENTS CLEARED	TOTAL TREATMENTS CURED
Smith/RE	70.0a	10.0a	1/1	0/1			1/1	0/1
	140.0a	20.0a			1/1	0/1	1/1	0/1
	70.0a	10.0a	1/2	0/2			1/2	0/2
	2.31b	0.33						
	70.0a	10.0	2/2	1/2	2/2	1/2	4/4	2/4
	7.0b	1.0						
	140.0a	20.0			1/1	1/1	1/1	1/1
	7.0b	1.0						

a. WR 99210

b. WR 139004, folinic acid

TABLE 10

DETAILED ACTIVITY OF ATTEMPTS TO INDUCE RESISTANCE TO WR 250417AG (BN 34278) IN VIETNAM SMITH/RE STRAIN INFECTIONS OF PLASMODIUM FALCIPARUM

Aotus No.	Daily Dose Mg/Kg	Parasitemia per cmm x 10^3										
		Day Pre-Rx	Day of Treatment			Day Post Treatment						
			1	2	3	1	2	3	4	5	6	7
12719	0.25	3	11	62	28	8	0.3	<0.01	<0.01	<0.01	0.5 (Inoc 12718)	
12720	0.25	4	19	147	151	189	370	(Inoc 12717)				
12719r	0.75	7	2	20	1	<0.01	0	0	0	0	0	0
12718	0.75	2	158	59	10	1	0.2	<0.01	0	0	<0.01	<0.01
12720r	0.75	189	370	154	103	6	2	1	<0.01	0	0	0 *
12717	0.75	4	22	15	16	0.9	<0.01	0	0	0	0	0
12721	2.5	0.8	2	9	5	0.3	<0.01	0	0	0	0	0
12722	2.5	2	10	24	13	0.5	<0.01	0	0	0	0	0
12718r	2.5	33	18	2	1	0.1	<0.01	<0.01	<0.01	0	0	0
12720rr	2.5	5	4	4	1	<0.01	0	0	0	0	0	0
12701	2.5	2	102	134	22	5	1	0.9	4	1	Re-Rx, higher dose	
12717r	2.5	3	5	2	0.3	<0.01	0	0	0	0	0	0
12721r	10.0	<0.01	0.9	2	0.4	<0.01	0	0	0	0	0	0
12722r	10.0	0	<0.01	0.4	<0.01	0	0	0	0	0	0	0
12720rrr	10.0	<0.01	0.3	<0.01	<0.01	<0.01	<0.01	<0.01	0	0	0	0
12701r	10.0	1	376	65	44	5	0.3	0.5	<0.01	<0.01	<0.01	<0.01
12721rr	20.0	<0.01	<0.01	<0.01	<0.01	0	0	0	0	0	0	0
12722rr	20.0	0.7	0.3	<0.01	<0.01	0	0	0	0	0	0	0

* INOC 12701 PRIOR TO RE-Rx

TABLE 11

SUMMARY OF ATTEMPTS TO INDUCE RESISTANCE TO WR 250417AG (BN 34278) IN VIETNAM SMITH/RE STRAIN INFECTIONS OF PLASMODIUM FALCIPARUM

Monkey No.	Daily Dose x 3 Mg/kg	Response of Parasitemia to Rx			Days from Initial Rx to parasite Clearance	Days from Final Rx To Recru-descence	Notes
		None	Suppressed	Cleared			
12719	0.25		+		n.a.	n.a.	Re-Rx, higher dose (INOC 12718)
12720	0.25		+		n.a.	n.a.	Re-Rx, higher dose (INOC 12717)
12719r	0.75			+	5		NEG 57 DAYS
12718	0.75		+		n.a.	n.a.	Re-Rx, higher dose
12720r	0.75			+	8	8	Re-Rx
12717	0.75			+	6	21	Re-Rx, higher dose
12721	2.5			+	6	12	Re-Rx, higher dose
12722	2.5			+	6	15	Re-Rx, higher dose
12718r	2.5				8		NEG 34 DAYS
12720rr	2.5			+	5	15	Re-Rx,higher dose(SUBINOC 12701)
12701	2.5		+				Re-Rx, higher dose
12717r	2.5			+	5		NEG. 29 DAYS
12721r	10.0			+	5	14	Re-Rx, higher dose
12722r	10.0			+	4	11	Re-Rx, higher dose
12720rrr	10.0			+	7		NEG. 26 DAYS
12701r	10.0			+	15	29	
12721rr	20.0			+	4		NEG 29 DAYS
12722rr	20.0			+	4		NEG 36 DAYS

TABLE 12

DETAILED ACTIVITY OF ATTEMPTS TO INDUCE RESISTANCE TO WR 169626AC (BK 09350) IN VIETNAM SMITH/RE INFECTIONS OF PLASMODIUM FALCIPARUM

Aotus No.	Daily Dose Mg/Kg	Parasitemia per cmm x 10^3										
		Day Pre-Rx	Day of Treatment			Day Post Treatment						
			1	2	3	1	2	3	4	5	6	7
12684	0.25	2	6	20	2	<0.01	<0.01	0	0	0	<0.01	(INOC 12681)
12686	0.25	1	1	2	0.4	<0.01	0	0	0	0	0	(INOC 12725)
12684r	0.75	2	2	3	2	<0.01	<0.01	<0.01	0	<0.01	<0.01	<0.01
12681	0.75	1	89	7	5	1	<0.01	0	0	0	0	(INOC 12674)
12686r	0.75	4	1	2	2	0.7	<0.01	0	0	0	0	0
12725	0.75	1	6	3	1	<0.01	0	0	0	0	0	0
12683	5.0	3	5	25	7	1	<0.01	0	0	0	0	0
12707	5.0	3	9	6	4	0.7	<0.01	0	0	0	0	0
12681r	5.0	111	180	51	2	<0.01	<0.01	0	0	0	0	0
12674	5.0	<0.01	3	0.7	0.3	<0.01	0	0	0	0	0	0
12725r	5.0	2	31	4	0.8	0.03	<0.01	<0.01	0	-	0	0
12681rr	10.0	2	5	2	0.3	<0.01						

TABLE 13

SUMMARY OF ATTEMPTS TO INDUCE RESISTANCE TO WR 169626AC (BK 09350) IN VIETNAM SMITH/RE STRAIN INFECTIONS OF PLASMODIUM FALCIPARUM

Monkey No.	Daily Dose x 3 Mg/kg	Response of Parasitemia to Rx			Days from Initial Rx to parasite Clearance	Days from Final Rx To Recru-descence	Notes
		None	Suppressed	Cleared			
12684	0.25			+	6	9	Re-Rx, higher dose (INOC 12681)
12686	0.25			+	5	16	Re-Rx, higher dose (INOC 12725)
12684r	0.75			+	16		NEG 45 DAYS
12681	0.75			+	6	9	Re-Rx, higher dose (INOC 12674)
12686r	0.75			+	5		NEG 45 DAYS
12725	0.75			+	5	12	Re-Rx, higher dose
12683	5.0			+	6		NEG 66 DAYS
12707	5.0			+	6		NEG 46 DAYS*
12681r	5.0			+	6	30	Re-Rx, higher dose
12674	5.0			+	5		NEG 33 DAYS
12725r	5.0			+	7		NEG 22 DAYS
12681rr	10.0						In Progress

* DIED - Intercurrent infection

TABLE 14

INFECTION PARAMETERS OF THE NEW GUINEA AMRU-2 (CQS) STRAIN OF PLASMODIUM VIVAX

Host Alteration (No.)	Patent Period Days Mean (Range)	Maximum Parasitemia/mm^3 Mean (Range)	No. of monkeys with recrudescences
Splenectomy (11)	29 (14 - 37)	24,006 (4,340 - 68,150)	2
None (3)	10 (0 - 25)	1,042 (0 - 3,820)	1

TABLE 15

INFECTION PARAMETERS OF THE NEW GUINEA AMRU-1 (CQR) STRAIN OF PLASMODIUM VIVAX

Host Alteration (No.)	Patent Period Days Mean (Range)	Maximum Parasitemia/mm^3 Mean (Range)	No. of Monkeys with recrudescences
Splenectomy (2)	70 (61 - 78)	39,830 (27,740 - 51,920)	2
None (13)	31 (19 - 52)	26,722 (9,140 - 47,630)	6

TABLE 16

DETAILED ACTIVITY OF WR 1544BM (AR 20613), CHLOROQUINE, AGAINST INFECTIONS OF THE NEW GUINEA AMRU-1 STRAIN OF PLASMODIUM VIVAX

Aotus No.	Daily Dose Mg/Kg	Parasitemia per cmm x 10^3									
		Day Pre-Rx	Day of Treatment							Day Post Treatment		
			1	2	3	4	5	6	7	1	2	3
12593	2.5	0.6	4	10	14	16	16	10	3	2	3	3
12600	2.5	0.5	2	8	16	26	14	20	29	11	8	20
12605	2.5	0.5	4	10	32	19	25	40	44	6	9	30
12199	5.0	1	5	23	13	32	11	10	13	5	11	5
12597	5.0	0.6	4	9	15	28	25	12	39	7	18	30
12606	5.0	0.4	2	6	14	33	21	8	42	10	10	33

TABLE 17

DETAILED ACTIVITY OF WR 1544BM (AR 20613), CHLOROQUINE, AGAINST INFECTIONS OF THE NEW GUINEA AMRU-1 STRAIN OF *PLASMODIUM VIVAX*

Aotus No.	Daily Dose Mg/Kg	Parasitemia per cmm x 10^3										
		Day Pre-Rx	Day of Treatment			Day Post Treatment						
			1	2	3	1	2	3	4	5	6	7
12592	10.0	0.6	4	9	13	17	18	21	44	19	32	57
12596	10.0	0.3	3	6	12	25	17	12	54	2	2	3
12601	10.0	0.4	5	8	11	17	22	37	63	12	23	46

TABLE 18

SUMMARY OF THE ACTIVITY OF WR 1544BM (AR 20613), CHLOROQUINE AGAINST INFECTION OF THE NEW GUINEA AMRU-1 STRAIN OF PLASMODIUM VIVAX

Monkey No.	Dose x 7 Mg/Kg	Response of Parasitemia to Rx			Days from Initial Rx to Parasite Clearance	Days from Final Rx To Recru-descence	Notes
		None	Suppressed	Cleared			
12593	2.5		+		22		Neg. 17 days
12600	2.5		+		28		Neg. 21 days
12605	2.5	+			26		Neg. 23 days
12199	5.0		+		32		Neg. 17 days
12597	5.0	+			32		Neg. 17 days
12606	5.0	+			30	37	

TABLE 19

SUMMARY OF THE ACTIVITY OF WR 1544BM (AR 20613), CHLOROQUINE, AGAINST INFECTIONS OF THE NEW GUINEA AMRU-1 STRAIN OF PLASMODIUM VIVAX

Monkey No.	Daily Dose x 3 Mg/Kg	Response of Parasitemia to Rx			Days from Initial Rx to Parasite Clearance	Days from Final Rx To Recru-descence	Notes
		None	Suppressed	Cleared			
12592	10.0	+			32		Neg. 17 days
12596	10.0	+			32	35	
12601	10.0	+			26		Neg. 23 days

TABLE 20

SUMMARY OF THE ACTIVITY OF WR 1544BM (AR 20613), CHLOROQUINE AGAINST PLASMODIUM VIVAX INFECTIONS

MALARIA STRAIN	DOSE mg/kg TOTAL	DOSE mg/kg DAILY	PRIMARY TREATMENTS CLEARED	PRIMARY TREATMENTS CURED	REPEAT TREATMENTS CLEARED	REPEAT TREATMENTS CURED	TOTAL TREATMENTS CLEARED	TOTAL TREATMENTS CURED
AMRU-1	17.5	2.5	0/3	0/3			0/3	0/3
	30.0	10.0	0/3	0/3			0/3	0/3
	35.0	5.0	0/3	0/3			0/3	0/3

FIGURE 1

POSSIBLE INDUCTION OF RESISTANCE TO WR 250417AG(BN 34278)

12719 0.25 mg/kg - Suppressed

↓

12718 0.75 mg/kg - Suppressed
12719r 0.75 mg/kg Cleared - neg. 57 days

12718r 2.5 mg/kg Cleared - neg. 34 days

12720 0.25 mg/kg - Suppressed

↓

12717 0.75 mg/kg - Cleared/Recrudescence

12717r 2.5 mg/kg Cleared - Neg. 29 days

12720r 0.75 mg/kg - Cleared/Recrudescence

↓

12701 2.5 mg/kg - Suppressed
12720rr 2.5 mg/kg Cleared/Recrudescence

12701r 10.0 mg/kg Cleared/Recrudescence - In Progress
12720rrr 10.0 mg/kg Cleared - neg. 26 days

FIGURE 2

POSSIBLE INDUCTION OF RESISTANCE TO WR 169626AC(BK 09350).

12684 0.25 mg/kg - Cleared/Recrudescence

↓

12681 0.75 mg/kg - Cleared/Recrudescence
12684r 0.75 mg/kg - Cleared - neg. 45 days

↓

12674 5.0 mg/kg - Cleared - Neg. 33 days
12681r 5.0 mg/kg - Cleared/Recrudescence
12681rr 10.0 mg/kg - In Progress

12686 0.25 mg/kg - Cleared/Recrudescence

↓

12725 0.75 mg/kg - Cleared/Recrudescence
12686r 0.75 mg/kg - Cleared - neg. 45 days

12725r 5.0 mg/kg Cleared - neg. 22 days

DEPARTMENT OF THE ARMY
U.S. ARMY MEDICAL RESEARCH AND MATERIEL COMMAND
504 SCOTT STREET
FORT DETRICK, MARYLAND 21702-5012

REPLY TO
ATTENTION OF:

MCMR-RMI-S (70-1y) 7 Feb 97

MEMORANDUM FOR Administrator, Defense Technical Information Center, ATTN: DTIC-OCP, Fort Belvoir, VA 22060-6218

SUBJECT: Request Change in Distribution Statement

1. The U.S. Army Medical Research and Materiel Command has reexamined the need for the limitation assigned to technical reports written for Contract Number DAMD17-91-C-1072. Request the limited distribution statement for Accession Document Numbers ADB214740, ADB198405, ADB210896, ADB183789, and ADB173254 be changed to "Approved for public release; distribution unlimited." These reports should be released to the National Technical Information Service.

2. Point of contact for this request is Mrs. Judy Pawlus at DSN 343-7322.

FOR THE COMMANDER:

GARY R. GILBERT
Colonel, MS
Deputy Chief of Staff for
Information Management

Completed
22 May 2000
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